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EXAMINER	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/669,976	ENGEL ET AL.	
	Examiner	Art Unit	
	STEPHANIE K. MUMMERT	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *22 October 2009*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,2,4-22 and 25-34* is/are pending in the application.

4a) Of the above claim(s) *17-22* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,2,4-16 and 25-34* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *10/22/09*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicant's amendment filed on October 22, 2009 is acknowledged and has been entered. Claim 3 and 23-24 have been canceled. Claims 29-34 have been added. Claims 1-2, 4-16 and 25-34 are pending. Claims 17-22 are withdrawn from consideration as being drawn to a non-elected invention.

All of the amendments and arguments have been thoroughly reviewed and considered but are not found persuasive for the reasons discussed below. Any rejection not reiterated in this action has been withdrawn as being obviated by the amendment of the claims. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Claims 1-2, 4-16 and 25-34 are discussed in this Office action.

This action is made FINAL as necessitated by New Claims.

Previous Grounds of Rejection

The objection to the specification as improperly incorporating subject matter is withdrawn in view of the amendment to the specification.

The statements of rejection have been updated to correct a typographical error in the patent number associated with Backus, consistent with the number provided on the references cited form.

The rejection below in view of Backus, Bustin and Birch now includes newly added claim 29.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

1. Claims 1-2, 4-11, 16 and 28-29 are rejected under 35 U.S.C. 103(a) as being unpatentable over Backus et al. (US Patent 5,705,366; January 1998) in view of Bustin, SA (Journal of Molecular Endocrinology, 2000, vol. 25, p. 169-193) and further in view of Birch et al. (US Patent 5,773,258; June 1998). Backus discloses a method of amplification of multiple target nucleic acids in the presence of a nonionic, polymeric volume exclusion agent (Abstract).

With regard to claim 1, Backus teaches a method for the coamplification of two or more target nucleic acids having different sequence compositions, said method comprising at least 15 primary amplification cycles (col. 2, line 67 to col. 3, line 3), each amplification cycle comprising the sequential steps of:

(A) heating a reaction mixture comprising two or more target nucleic acids and a hot start DNA polymerase, or their primer extension products, at a first temperature, T1, for denaturation of the strands of the target nucleic acids or their primer extension products (col. 3, lines 4-8; col. 7, lines 11-19, where the inclusion of a polymerase and the inclusion of an antibody specific to the

DNA polymerase which inhibits enzymatic activity below about 50°C and which is inactivated at higher temperatures), and

(B) priming the denatured strands with a set of unmodified or modified primers specific to and hybridizable with opposing strands of each target nucleic acid to be amplified, by cooling to a second temperature, T2 (col. 3, lines 9-12), and

(C) either as a continuation of step (B) or in a separate step, forming primer extension products in a reaction mixture of PCR reagents, by incubation at a third temperature, T3, provided that when priming and primer extension product formation are carried out in the same step, T2 and T3 are the same (col. 3, lines 13-18),

(D) in the course of the reaction in each amplification cycle or in an amplification cycle after the last primary amplification cycle, detecting two or more of the primer extension products having different sequence composition as an indication of coamplification of the target nucleic acids (col. 3, lines 46-48); an improvement comprising using the DNA polymerase included in the reaction mixture of step (A), a modified thermostable hot start DNA polymerase enzyme that becomes active only after incubation at temperatures above 50oC (col. 3, lines 4-8; col. 7, lines 11-19, where the inclusion of a polymerase and the inclusion of an antibody specific to the DNA polymerase which inhibits enzymatic activity below about 50°C and which is inactivated at higher temperatures), and using in at least one of the primary amplification cycles, 1 to 20 weight % of a nonionic polymeric volume exclusion agent (col. 3, lines 19-21, where the disclosed percentage of 4 weight % falls within the range(s) claimed).

With regard to claim 2, Backus teaches an embodiment of claim 1, wherein the amount of polymeric volume exclusion agent in said reaction mixture is 1 to 15 weight % (col. 3, lines 19-21, where the disclosed percentage of 4 weight % falls within the range(s) claimed).

With regard to claim 4, Backus teaches an embodiment of claim 1, wherein the amount of polymeric volume exclusion agent in said reaction mixture is 1 to 8 weight % (col. 3, lines 19-21, where the disclosed percentage of 4 weight % falls within the range(s) claimed).

With regard to claim 5, Backus teaches a method according to one of claims 1, 2 or 4, characterized in that the volume exclusion agent is selected from the group consisting of a polyether, a reaction product of a sugar with epichlorohydrin, a polysaccharide, and a polyacrylate (col. 7, lines 36-41).

With regard to claim 6, Backus teaches a method according to claim 5, characterized in that the volume exclusion agent is selected from the group of polyethers of the general formula;

$$H\text{-}(\text{-}O\text{-}R\text{-})_n\text{-}H$$

wherein R is an alkylene bridge of 1 to 6 carbon atoms - branched or unbranched - and n is an integer of 15 to 1000 (col. 3, lines 42-48).

With regard to claim 7, Backus teaches an embodiment of claim 6, characterized in that R may represent l,2-ethylene, l,3-propylene, l,2-propylene, 2-hydroxy-l,3-propylene, 3-hydroxy-l,2-propylene, l,4-butylene, l,3-butylene, or l,2-hexylene (col. 7, lines 48-52).

With regard to claim 8, Backus teaches an embodiment of claim 6, characterized in that the polyether is poly(ethylene glycol) (col. 7, lines 53-56, where it is noted that a preferred R group is polyethylene glycol).

With regard to claim 9, Backus teaches an embodiment of claim 8, characterized in that

the polytethylene glycol) has a molecular weight in the range of 1000 daltons to 2,000,000 daltons (col. 7, lines 59-67, where it is noted that the preferred range is between about 1000 to about 20,000, wherein the term about represents +/-10% and wherein the preferred range of molecular weights falls within the range as claimed, presuming that it is intended for the claimed ranges to be measured in daltons; col. 15, lines 19-21, where PEG-8000, a polyethylene glycol with a molecular weight of 8000 daltons was used in the examples).

With regard to claim 10, Backus teaches an embodiment of claim 8, characterized in that the poly(ethylene glycol) has a molecular weight in the range of 3000 daltons to 500,000 daltons (col. 7, lines 59-67, where it is noted that the preferred range is between about 1000 to about 20,000, wherein the term about represents +/-10% and wherein the preferred range of molecular weights falls within the range as claimed, presuming that it is intended for the claimed ranges to be measured in daltons; col. 15, lines 19-21, where PEG-8000, a polyethylene glycol with a molecular weight of 8000 daltons was used in the examples).

With regard to claim 11, Backus teaches an embodiment of claim 8, characterized in that the poly(ethylene glycol) has a molecular weight of about 8000 daltons (col. 7, lines 59-67, where it is noted that the preferred range is between about 1000 to about 20,000, wherein the term about represents +/-10% and wherein the preferred range of molecular weights falls within the range as claimed, presuming that it is intended for the claimed ranges to be measured in Daltons; col. 15, lines 19-21, where PEG-8000, a polyethylene glycol with a molecular weight of 8000 daltons was used in the examples).

With regard to claim 16, Backus teaches an embodiment of claim 5, characterized in that the polyacrylate is selected from the group consisting of poly(hydroxyethyl acrylate) or poly(2,3-

dihydroxypropyl acrylate) (col. 8, lines 12-15).

With regard to claim 29, Backus teaches an embodiment of claim 1 wherein each set of primers hybridizable with opposing strands of each target nucleic acid to be amplified is different (col. 5, lines 63-67, where the primers are disclosed as hybridizable with opposing strands of the target, see also col. 13, lines 29-38, where sequences of primers are provided for amplification of HIV and beta globin and the primers are each different).

Regarding claims 1, 2 and 4, while Backus teaches a reversibly modified thermostable DNA polymerase, Backus does not teach a modification that comprises a chemical modification as established in the specification. Birch teaches the reversible modification of DNA polymerase by an inhibiting agent (Abstract).

With regard to claims 1, 2 and 4, Birch teaches a chemically-modified thermostable hot start DNA polymerase enzyme that becomes active only after incubation at temperatures above 50°C (Abstract; col. 4, lines 49-58, where the reversibly inactivated enzyme is a thermostable DNA polymerase; col. 3, lines 1-19, where a DNA polymerase is reversibly inactivated using treatment with a modifier reagent and becomes active at a temperature of about 50 °C, col. 3, lines 44-51).

Furthermore, regarding claims 1, 2 and 4, neither Backus nor Birch explicitly teach that the two or more target nucleic acids are present at comparable copy numbers whereas the maximum difference between the lowest and the highest copy number is 10 fold. Regarding claims 2 and 4, Backus does not teach that the reaction mixture comprises a sequence specific probe which binds within the primer binding regions and which generates a fluorescent signal after hybridization.

Bustin teaches an overview of the quantitation of mRNA using a variety of methods, including quantitative real-time RT-PCR, a method which incorporates a variety of means of detection, including hybridization probes (Abstract).

With regard to claims 1-2 and 4, Bustin teaches the coamplification of nucleic acids which are present at comparable copy numbers, wherein the maximum difference between the lowest and highest copy number is 10-fold (p. 182, col. 1, 2nd paragraph, where the normalization of quantification of a target nucleic acid is accomplished through co-amplification of an internal control target sequence, referred to as an endogenous control. It is also noted that the endogenous control should be expressed at roughly the same level as the RNA under study; see also p. 185, 'multiplex RT-PCR' heading, where multiple primer sets are used to amplify multiple specific targets simultaneously).

With regard to claim 1, Bustin discloses a sequence specific probe which binds within the primer binding regions and which generates a fluorescent signal after hybridization (Figure 3 and p. 174, where molecular beacon probes were described; Figure 4A-C right side and p. 177, where 'hybdrization probes' were described; Figure 5, p. 177, where TaqMan hydrolysis probes were described).

With regard to claim 28, Bustin teaches an embodiment of claim 1, characterized in that the sequence specific labeled probe is fluorescently labeled (Figure 3 and p. 174, where molecular beacon probes were described and are fluorescently labeled; Figure 4A-C right side and p. 177, where 'hybdrization probes' were described; Figure 5, p. 177, where TaqMan hydrolysis probes were described).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have extended the teachings of Backus to incorporate the fluorescent hybridization probes and coamplification of a target sequence of comparable copy number of Bustin, to arrive at the claimed invention with a reasonable expectation for success. As taught by Bustin, "The accepted method for minimizing these errors and correcting for sample-to-sample variation is to amplify, simultaneously with the target, a cellular RNA that serves as an internal reference against which other RNA values can be normalized. The ideal internal standard should be expressed at a constant level among different tissues of an organism, at all stages of development, and should be unaffected by the experimental treatment. In addition, an exogenous control should also be expressed at roughly the same level as the RNA under study" (p. 182, col. 1). Therefore, one of ordinary skill in the art at the time the invention was made would have been motivated to extend the coamplification in the presence of a polymeric volume exclusion agent taught by Backus to incorporate the teachings of Bustin with a reasonable expectation for success in order to achieve quantitative coamplification of two nucleic acid targets of similar copy number.

Furthermore, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have extended the teachings of Bustin and Backus to include the chemical modification through reaction with an chemical as taught by Birch to arrive at the claimed invention with a reasonable expectation for success. The reversible modification taught by Backus includes "an antibody specific to the DNA polymerase, which antibody inhibits enzymatic activity at a temperature below about 50°C, but which antibody is deactivated at higher temperatures" (col. 7, lines 11-20). In a related technique, Birch teaches methods that

"use a reversibly inactivated thermostable enzyme which can be reactivated by incubation in the amplification reaction mixture at an elevated temperature" (col. 2, lines 62-65). Birch also teaches a preferred embodiment wherein "the amplification reaction is a polymerase chain reaction (PCR) and a reversibly-inactivated thermostable DNA polymerase is used. The reaction mixture is incubated prior to carrying out the amplification reaction at a temperature which is higher than the annealing temperature of the amplification reaction. Thus, the DNA polymerase is inactivated until the temperature is above the temperature which insures specificity of the amplification reaction, thereby reducing non-specific amplification" (col. 4, lines 49-58). Both Backus and Birch teach modification that is reversible with an increase in temperature. Therefore, one of ordinary skill in the art at the time the invention was made would have been motivated to have extended the teachings of Bustin and Backus to include the chemical modification through reaction with a chemical as taught by Birch to arrive at the claimed invention with a reasonable expectation for success.

2. Claims 12-15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Backus et al. (US Patent 5,705,366; January 1998) in view of Bustin, SA (Journal of Molecular Endocrinology, 2000, vol. 25, p. 169-193) and Birch et al. (US Patent 5,773,258; June 1998) as applies to claims 1-2, 4-11, 16 and 28-29 and further in view of Reed et al. (US Patent 5,459,038; October 1995) and Demke et al. (Biotechniques, 1992, vol. 12, no. 3, p. 333-334). Backus discloses a method of amplification of multiple target nucleic acids in the presence of a nonionic, polymeric volume exclusion agent (Abstract).

Regarding claims 12-15, while Backus teaches amplification in the presence of Dextran sulfate as inhibiting to amplification, Demke provides an explanation that while dextran sulfate is inhibitory to PCR amplification, Dextran does not inhibit amplification via PCR.

Demke does not provide explicit teaching that dextran provides an improvement to PCR amplification without the inclusion of a volume exclusion agent. Reed teaches amplification of samples in the presence of Dextran (col. 19, lines 9-26, where the inclusion of Dextran results in more efficient amplification).

With regard to claim 12, Reed teaches an embodiment of claim 5, characterized in that the volume exclusion reagent is a dextran (col. 19, lines 9-26, where the inclusion of Dextran results in more efficient amplification).

With regard to claim 13, Reed teaches an embodiment of claim 12, characterized in that the dextran has a molecular weight in the range of 1000 to 2,000,000 (col. 16, lines 10-20, where the dextran is T500, which comprises a MW of 500,000).

With regard to claim 14, Reed teaches an embodiment of claim 12, characterized in that the dextran has a molecular weight in the range of 3000 to 500,000 (col. 16, lines 10-20, where the dextran is T500, which comprises a MW of 500,000).

With regard to claim 15, Reed teaches an embodiment of claim 12, characterized in that the dextran has a molecular weight in the range of 40,000 daltons to 60,000 daltons (col. 16, lines 10-20, where the dextran is T500, which comprises a MW of 500,000, see obviousness rejection below).

While Reed teaches a Dextran of molecular weight 500,000, Reed also teaches that dextran generally provides an improvement over PCR amplification reactions that are not

conducted in the presence of dextran. Thus, an ordinary practitioner would have recognized that the results optimizable variables of time, concentration and product amount could be adjusted to maximize the desired results. As noted in *In re Aller*, 105 USPQ 233 at 235,

> More particularly, where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation.

Routine optimization is not considered inventive and no evidence has been presented that the results were other than routine, that the products resulting from the optimization have any unexpected properties, or that the results should be considered unexpected in any way as compared to the closest prior art.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have extended the teachings of Backus to incorporate the fluorescent hybridization probes and coamplification of a target sequence of comparable copy number the of Bustin, to arrive at the claimed invention with a reasonable expectation for success. As taught by Bustin, "The accepted method for minimizing these errors and correcting for sample-to-sample variation is to amplify, simultaneously with the target, a cellular RNA that serves as an internal reference against which other RNA values can be normalized. The ideal internal standard should be expressed at a constant level among different tissues of an organism, at all stages of development, and should be unaffected by the experimental treatment. In addition, an exogenous control should also be expressed at roughly the same level as the RNA under study" (p. 182, col. 1). Therefore, one of ordinary skill in the art at the time the invention was made would have been motivated to extend the coamplification in the presence of a polymeric volume exclusion agent taught by Backus to incorporate the teachings of Bustin with a reasonable expectation for

success in order to achieve quantitative coamplification of two nucleic acid targets of similar copy number.

Furthermore, it would have been prima facie obvious in view of the teachings of Demke and Reed to include dextran into the method of amplification taught by Backus in view of Bustin. First, it is noted that Backus teaches amplification in the presence of PEG and dextran sulfate. While Backus teaches that dextran sulfate is inhibitory to amplification, Demke teaches "the inhibitory nature of some polysaccharides with free acidic groups is further demonstrated by contrasting dextran and dextran sulfate. Dextran (neutral) has no interfering effects at 500:1 ratio, wheras dextran sulfate was very inhibitory (Table 1). Therefore, considering the teachings of Demke, it would have been prima facie obvious to substitute the dextran sulfate taught by Backus for the equivalent dextran as taught by Demke. Furthermore, as taught by Reed, "the inclusion of polysaccharide dextran (or similar) results in three unique advantages: firstly, its inclusion results in more efficient amplification leading to markedly higher sensitivity and specificity" (col. 19, lines 9-26). Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have extended the method taught by Backus to include dextran as taught by Reed and Demke to achieve efficient amplification with higher sensitivity and specificity with a reasonable expectation for success.

3. Claim 25 is rejected under 35 U.S.C. 103(a) as being unpatentable over Backus et al. (US Patent 5,705,366; January 1998) in view of Bustin, SA (Journal of Molecular Endocrinology, 2000, vol. 25, p. 169-193) and Birch et al. (US Patent 5,773,258; June 1998) as applied to claims 1-2, 4-11, 16 and 28-29 above, and further in view of Ivanov et al. (US Patent 6,183,998;

February 2001). Backus discloses a method of amplification of multiple target nucleic acids in the presence of a nonionic, polymeric volume exclusion agent (Abstract).

Backus in view of Bustin and Birch renders obvious claims 1-2, 4-11, 16 and 28 as recited in the 103 rejection stated above. While Backus in view of Bustin and Birch teaches a chemically modified DNA polymerase, neither Backus, Bustin or Birch teaches that the modification is due to a reaction with an aldehyde. Ivanov teaches reversible modification of DNA polymerases through reaction with an aldehyde (Abstract).

With regard to claim 25, Ivanov teaches an embodiment of claim 1, 2 or 4, wherein said chemically modified DNA polymerase is modified by reaction with an aldehyde (Abstract).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have extended the teachings of Bustin and Backus to include the chemical modification through reaction with an aldehyde as taught by Ivanov to arrive at the claimed invention with a reasonable expectation for success. The reversible modification taught by Backus includes "an antibody specific to the DNA polymerase, which antibody inhibits enzymatic activity at a temperature below about 50°C, but which antibody is deactivated at higher temperatures" (col. 7, lines 11-20). In a related technique, Ivanov teaches "for reversible inactivation of thermostable enzymes using a chemical modification under essentially aqueous conditions. In particular, the thermostable enzymes of the present invention are reversibly modified in the presence of an aldehyde". Furthermore, Ivanov teaches "enzymatic activity of the present chemically modified enzymes is increased at least two-fold within thirty minutes when incubated at a more elevated temperature, i.e. above 50 °C, preferably at a temperature of 75 °C to 100 °C" (col. 3, lines 1-14). Both Backus and Ivanov teach modification that is

reversible with an increase in temperature. Therefore, one of ordinary skill in the art at the time the invention was made would have been motivated to have extended the teachings of Bustin and Backus to include the chemical modification through reaction with an aldehyde as taught by Ivanov to arrive at the claimed invention with a reasonable expectation for success.

New Grounds of Rejection as necessitated by Amendment

Information Disclosure Statement

The information disclosure statement (IDS) submitted on October 22, 2009 was filed in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

4. Claims 26-27 are rejected under 35 U.S.C. 103(a) as being unpatentable over Backus et al. (US Patent 5,705,366; January 1998) in view of Bustin, SA (Journal of Molecular Endocrinology, 2000, vol. 25, p. 169-193) and Birch et al. (US Patent 5,773,258; June 1998) as applied to claims 1-2, 4-11, 16 and 28-29 above and further in view of Mansfield et al. (Molecular Cellular Probes, 1995, vol. 9, p. 145-156). Backus discloses a method of

amplification of multiple target nucleic acids in the presence of a nonionic, polymeric volume exclusion agent (Abstract).

5. Backus in view of Bustin and Birch render obvious all of the limitations of claims 1-2, 4-11, 16 and 28. However, neither Backus, Bustin or Birch teach that one of the primers is fluorescently labeled or that one of the primers is labeled with a specific binding moiety. Mansfield teaches a variety of primer labeling techniques (Abstract).

With regard to claim 26, Mansfield teaches an embodiment of claim 1, characterized in that one of the primers of each primer set is fluorescently labeled (p. 145, col 2; p. 146, col. 1, where PCR incorporating a 5' end tagged primer sequence labeled with a fluorescent nucleotide is described).

With regard to claim 27, Mansfield teaches an embodiment of claim 1, characterized in that one of the primers of each primer set is labeled with a specific binding moiety (p. 145, col 2, where a small molecule like fluorescein, biotin or digoxigenin can be incorporated into the detection reagent; Figure 1, where the biotin label is attached to a probe, but the attachment of a probe or a primer is equivalent).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have adjusted the teachings of Backus, Bustin and Birch to include the labeled primers of Mansfield to arrive at the claimed invention with a reasonable expectation for success. As taught by Mansfield, "fluorescent, chemiluminescent, bioluminescent and colorimetric approaches have been used as alternatives to radioactive detection methods. Fluorogenic or chemiluminescent or chemiluminescent substrates allow subattomole level detection of DNA labels in solid-phase membrane-based hybridization assays with much greater

sensitivity than using colorimetric methods". Mansfield also emphasizes that "fluorescence detection is by far the most versatile" (p. 154, col. 1). Therefore, one of ordinary skill in the art at the time the invention was made would have been motivated to have adjusted the teachings of Backus, Bustin and Birch to include the labeled primers of Mansfield to achieve sensitive detection of amplification products.

6. Claims 30-34 are rejected under 35 U.S.C. 103(a) as being unpatentable over Backus et al. (US Patent 5,705,366; January 1998) in view of Bustin, SA (Journal of Molecular Endocrinology, 2000, vol. 25, p. 169-193) and Birch et al. (US Patent 5,773,258; June 1998) as applied to claims 1-2, 4-11, 16 and 28-29 above and further in view of Grondahl et al. (J. Clin. Micro., 1999, 37(1):1-7) . Backus discloses a method of amplification of multiple target nucleic acids in the presence of a nonionic, polymeric volume exclusion agent (Abstract). Backus in view of Birch and Bustin render obvious the limitations of claims 1-2, 4-11, 16 and 28-29. However, neither Backus, Birch nor Bustin teach the simultaneous amplification of six or eight targets or more.

With regard to claim 30, Grondahl teaches an embodiment of claim 1, wherein the method is capable of coamplifying six (6) or eight (8) different target nucleic acids (p. 2, col. 1, where the primers for the nine different target nucleic acids are provided; Further, see abstract and title, where it is clear that multiplex amplification is carried out in a single tube and so at least nine targets are coamplified).

With regard to claim 32-33, Grondahl teaches an embodiment of claim 1, comprising six (6) or eight (8) different target nucleic acids (p. 2, col. 1, where the primers for the nine different

target nucleic acids are provided; Further, see abstract and title, where it is clear that multiplex amplification is carried out in a single tube and so at least nine targets are coamplified).

With regard to claim 34, Grondahl teaches an embodiment of claim 1, comprising eight (8) sets of primers (p. 2, col. 1, where the primer sets for the nine different target nucleic acids are listed).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have adjusted the teachings of Backus, Bustin and Birch to include the level of multiplex as taught by Grondahl to arrive at the claimed invention with a reasonable expectation for success. As taught by Grondahl, "A multiplex reverse transcription-PCR (RT-PCR) assay was developed to allow in one test the detection of nine different microorganisms (enterovirus, influenza A and B viruses, respiratory syncytial virus [RSV], parainfluenzaviruses type 1 and type 3, adenovirus, Mycoplasma pneumoniae, and Chlamydia pneumoniae) that do not usually colonize the respiratory tracts of humans but, if present, must be assumed to be the cause of respiratory disease." (Abstract). This method is designed to overcome "the disadvantage of requiring different and time-consuming assay conditions for each organism detected and the use of several tubes for one sample, thus enlarging the risk of cross contamination" (p. 1, col. 2). Therefore, as Grondahl clearly teaches an improvement provided by multiplex coamplification carried out in a single tube, one of ordinary skill in the art at the time the invention was made would have been motivated to have adjusted the teachings of Backus, Bustin and Birch to include the higher level of simultaneous multiplex taught by Grondahl to arrive at the claimed invention with a reasonable expectation for success.

Response to Arguments

Applicant's arguments filed November 4, 2009 have been fully considered but they are not persuasive.

Applicant traverses the rejection of claims 1-2, 4-11, 16, 23 and 24 under Backus in view of Bustin and Birch. Applicant traverses on the grounds that the combination of references fails to support a case of obviousness due to no reasonable expectation for success and because Applicant believes there is evidence of unexpectedly superior results. Regarding the lack of a reasonable expectation for success, Applicant then argues this is an "obvious to try" situation. The legal standard for "reasonable expectation of success" is provided by case law and is summarized in MPEP 2144.08, which notes "obviousness does not require absolute predictability, only a reasonable expectation of success; i.e., a reasonable expectation of obtaining similar properties. See, e.g., In re O'Farrell, 853 F.2d 894, 903, 7 USPQ2d 1673, 1681 (Fed. Cir. 1988)." In this factual case, there is express suggestion in the prior art that directly points to the use of particular percentages of nonionic volume exclusion agent in the coamplification of two or more target nucleic acids. There is further express teaching in Bustin that the coamplification of targets present at similar copy number is preferred when the coamplified target is used as an internal control. This is sufficient for a reasonable expectation of success. The MPEP cites In re O'Farrell, which notes regarding "obvious to try" at page 1682, that,

> "In some cases, what would have been "obvious to try" would have been to vary all parameters or try each of numerous possible choices until one possibly arrived at a successful result, where the prior art gave either no indication of which parameters were critical or no direction as to which of many possible choices is

> likely to be successful. E.g ., In re Geiger , 815 F.2d at 688, 2 USPQ2d at 1278; Novo Industri A/S v. Travenol Laboratories, Inc ., 677 F.2d 1202, 1208, 215 USPQ 412, 417 (7th Cir. 1982); In re Yates , 663 F.2d 1054, 1057, 211 USPQ 1149, 1151 (CCPA 1981); In re Antonie , 559 F.2d at 621, 195 USPQ at 8-9. In others, what was "obvious to try" was to explore a new technology or general approach that seemed to be a promising field of experimentation, where the prior art gave only general guidance as to the particular form of the claimed invention or how to achieve it. In re Dow Chemical Co., 837 F.2d, 469, 473, 5 USPQ2d 1529, 1532 (Fed. Cir. 1985); Hybritech, Inc. v. Monoclonal Antibodies, Inc ., 802 F.2d 1367, 1380, 231 USPQ 81, 90-91 (Fed. Cir. 1 986), cert. denied, 107 S.Ct. 1606 (1987); In re Tomlinson ; 363 F.2d 928, 931, 150 USPQ 623, 626 (CCPA 1966).

The court in O'Farrell then, affirming the rejection, notes "Neither of these situations applies here." For the instant case, it is clear that neither situation applies here either. This is not a situation where the prior art suggests varying a variety of parameters, since the prior art directly points to the use of particular percentages of nonionic volume exclusion agent in the coamplification of two or more target nucleic acids and including a chemically modified "hot start polymerase" as claimed, a combination of elements which is specifically taught as mediating efficient amplification of target nucleic acids. The art applied in the instant rejection, Backus together with Birch, teach all of the elements of the claimed invention, except for a specific teaching of the coamplification of targets present in the same copy number. However, as taught by Bustin, the coamplification of targets present at similar copy number would have been obvious given the specific guidance in the prior art. Therefore, this is also not a situation where only general guidance was given. The prior art provides specific guidance directing the use of the coamplification of targets present at similar copy number (Bustin), while Backus and Birch point directly to the use of particular percentages of nonionic volume exclusion agents in the coamplification of two or more target nucleic acids and including a chemically modified "hot

start polymerase" as claimed, a combination of elements which is specifically taught as mediating efficient amplification of targets.

Also, while Applicant's arguments regarding the "design and execution of multiplex polymerase chain reactions" and that it is "anything but a straightforward undertaking" (p. 10 of remarks) are noted, the argument that the process of multiplex amplification of multiple targets does not provide evidence of a lack of reasonable expectation for success. While the citation to additional references which highlight the elements that must be optimized for successful multiplex amplification are appreciated, again it is noted that Applicant is effectively arguing that multiplex amplification requires optimization. A need for optimization of a multiplex amplification is not unexpected to one of ordinary skill and does not provide any basis for a lack of reasonable expectation for success. One of ordinary skill would understand the factors that need to be adjusted to optimize any type of multiplex amplification.

Next, Applicant argues that "Bustin fails to provide any guidance as to how to conduct multiplex PCR in such a context" and argue that Bustin merely cites to Karge. Applicant then makes another grand statement that "in 2002, the skilled artisan would had to go down the never trivial path of subtly adjusting a large number of known variables including concentration, sequence and GC-content of primers…" and "even more daunting for the skilled artisan would have been the unknown variables". Applicant also argues that amplification of "nucleic acids present at comparable copy numbers amounted to throwing metaphorical darts at a board filled with combinatorial prior art possibilities" (p. 12 of remarks).

These arguments are not persuasive because, contrary to Applicant's argument, Bustin does provide sufficient guidance regarding "how to conduct multiplex PCR", referring to the amplification of internal control nucleic acid, which is expressed at a constant level, or by looking to other house-keeping genes, including GAPDH, b-actin and rRNA, for example. The

citation to additional references provides specific guidance regarding the manner of how to conduct multiplex PCR. Therefore, these arguments are not persuasive. Applicant's arguments regarding the "daunting" task of optimization of multiplex amplification are not persuasive. Again it is reiterated that arguing that a method requires optimization is not evidence of a lack of reasonable expectation for success. A reasonable expectation does not require a guarantee of success.

Applicant also argues that "there is no evidence to suggest that the skilled artisan would have deemed the presence or absence of volume exclusion agents to effect the amplification of two or more different target nucleic acids present at comparable copy numbers any more or less than any other adjuvant". Applicant concludes "without having some specific reason as to why the skilled artisan would have used a volume exclusion agent in the amplification of two or more different target nucleic acids present at comparable copy numbers, the skilled artisan would have deemed an inclusion the equivalent of tossing another dart" (p. 14 of remarks).

These arguments have again been considered but are not persuasive. While Applicant continues to argue that there is no evidence that one of ordinary skill would look to the use of volume exclusion agents for the amplification of targets at comparable copy number, Applicant has provided no evidence that targets present at comparable copy number would not have a reasonable expectation for success. Applicant's "evidence" of the daunting effort of multiplex have been directed to multiplex amplification in general and generally of a higher multiplex and do not appear to address the issue of amplification of targets present at similar copy number. In the absence of evidence that shows that one of ordinary skill would have some reason to suspect a lack of a reasonable expectation for successful amplification of targets at comparable copy number, one would look to the positive teaching of Backus for "evidence" that choosing to include a volume exclusion agent is more than throwing a dart at a dartboard. Backus

specifically teaches "the advantages of the invention are achieved by including a water-soluble or water-swellable, non-ionic, polymeric volume exclusion agent within the amplification reaction" and that "the presence of this agent effectively allows the user to reduce the amount of primer needed for efficient amplification of the nucleic acids, which reduction then allows manipulation of the procedure to procedure so one nucleic acid is amplified preferentially". Backus also concludes that the volume exclusion agent "allows manipulation of the coamplification of multiple target nucleic acids even further" (col. 4, lines 30-47). The cited passage clearly indicates that Backus chooses to manipulate the amplification reaction to coamplify targets that are present in disparate concentrations and also that the process can be manipulated to achieve other results and types of coamplification. This passage indicates that the technique of including the volume-exclusion agent allows flexibility in amplification design and **does not** in any way indicate that the method is only applicable to targets that are present at 100,000 fold difference in concentration. Instead, Backus leaves room for other embodiments of coamplification of multiple targets. Therefore, Applicant's arguments regarding Backus are wholly unpersuasive.

Finally, Applicant argues evidence of unexpected results. However, other than arguments highlighting the apparent teaching of Applicant's specification, Applicant does not provide evidence that highlights why these results would be considered "unexpected" when compared to the prior art. It is also noted that Applicant's argument that the method "allows the skilled artisan to conduct a multiplex PCR assay in which at least six (6) if not eight (8), target sequences" were coamplified is arguing a feature that is not claimed in the independently claimed invention. Therefore, Applicant's arguments are not persuasive and the rejections are maintained.

Conclusion

No claims are allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to STEPHANIE K. MUMMERT whose telephone number is (571)272-8503. The examiner can normally be reached on M-F, 9:00-5:30. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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